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Restatement of Order Parameters in Biomembranes: Calculation of C-C Bond Order Parameters from C-D Quadrupolar Splittings

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ABSTRACT An expression for the C-C bond order parameter, S^{CC}, of membrane hydrocarbon chains has been derived from the observed C-D bond order parameters. It allows calculation of the probability of each of the C-C bond rotamers and, consequently, the number of *gauche* defects per chain as well as their projected average length onto the bilayer normal, thus affording the calculation of accurate hydrophobic bilayer thicknesses. The effect of temperature has been studied on dilauroyl-, dimyristoyl-, and dipalmitoylphosphatidylcholine (DLPC, DMPC, DPPC) membranes, as has the effect of cholesterol on DMPC. The salient results are as follows: 1) an odd-even effect is observed for the S^{CC} versus carbon position, k, whose amplitude increases with temperature; 2) calculation of S^{CC}, from nonequivalent deuterons on the *sn*-2 chain of lipids, S_2^{CC}, leads to negative values, indicating the tendency for the C_1-C_2 bond to be oriented parallel to the bilayer surface; this bond becomes more parallel to the surface as the temperature increases or when cholesterol is added; 3) calculation on the *sn*-2 chain length can be performed from C_1 to C_n, where n is the number of carbon atoms in the chain, and leads to 10.4, 12.2, and 13.8 Å for DLPC, DMPC, and DPPC close to the transition temperature, T_C, of each of the systems and to 9.4, 10.9, and 12.6 for $T - T_C = 30$–$40°C$, respectively; 4) separation of intra- and intermolecular motions allows quantitation of the number of *gauche* defects per chain, which is equal to 1.9, 2.7, and 3.5 for DLPC, DMPC, and DPPC near T_C and to 2.7, 3.5, and 4.4 at $T - T_C = 30$–$40°C$, respectively. Finally, the validity of our model is discussed and compared with previously published models.

INTRODUCTION

The concept of order parameter is now widely used in the world of liquid crystals. In biomembranes, it has also been introduced (Seelig and Niederberger, 1974; Seelig, 1977; Davis, 1983) to provide a basis for understanding the concept of membrane fluidity.

More recently, it has been proposed that one may separate ordering information according to the time scale at which the time and space averaging takes place (Petersen and Chan, 1977; Paddy et al., 1981). Intra- and intermolecular and collective order parameters or amplitudes of fluctuation thus have been defined. In favorable cases, the bilayer elastic constant can be measured (Meier et al., 1986; Mayer et al., 1988; Dufourc et al., 1992; Auguste et al., 1994). The relative ease in deuterating molecules and using solid state NMR has led to the appearance of so-called C-D bond order parameter profiles, i.e., the minute description of ordering of the membrane hydrophobic interior. This C-D bond profile has been proposed by Seelig and co-workers as a measure of bilayer hydrophobic thickness (Seelig and Seelig, 1974; Seelig and Niederberger, 1974; Schindler and Seelig, 1975). This approach relies on the calculation of the average length of a saturated hydrocarbon chain spanning half of the membrane interior from the various C-D bond conformers in rapid exchange at each carbon position along the chain.

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Although Seelig and co-workers made a fantastic breakthrough in understanding membrane ordering, their original approach did not allow separation of motions in terms of segment conformations and molecule wobbling, which have been demonstrated to contribute to the averaging detected by solid state NMR (Petersen and Chan, 1977; Paddy et al., 1981; Meier et al., 1986). Because lipid chains can only be deuterated from C_2 to C_n, the full length (C_1 to C_n) clearly was underestimated by the direct use of C-D order parameters. The model used for chain length calculation was based on rapid exchange between three conformations of D-C-D planes with respect to the bilayer normal. As will be seen herein, the most general way to describe conformational disorder at a given methylene group in a aliphatic chain is to consider four conformers (rotations around C-C bonds). In addition, when using deuterium NMR of *sn*-2 chains, there is nonequivalence of deuterons at position C_2 (Seelig and Seelig, 1974; Engel and Cowburn, 1981); this point is never taken into account in the literature when calculating chain length.

By a proper separation of inter- and intramolecular contributions, one should be able to calculate the probability of *gauche* defects at a given chain segment and for the entire chain. These occur at the picosecond time scale and are usually detected by infrared spectroscopy. As we will see herein, this information can also be obtained from NMR.

The aim of the paper is to attempt to circumvent the drawbacks pointed above and to give a general approach for calculation of chain length (including nonequivalence of C-D bonds) and conformational defects. We introduce the C-C order parameter, which can be used with ^2H- or ^{13}C-NMR of labeled chains. This concept allows calculation of the intramolecular order parameters and conformer probabilities,

which are very sensitive to sterical and dynamical variations of the system. Examples are provided with dimyristoylphosphatidylcholine (DMPC) in the presence or absence of cholesterol, and dilauroyl- and dipalmitoylphosphatidylcholine (DLPC, DPPC).

MATERIALS AND METHODS

Dilauroyl-, dimyristoyl- and dipalmitoylphosphatidylcholine, perdeuterated on their *sn*-2 fatty acyl chain or specifically labeled, were either purchased from Avanti Polar Lipids (Alabaster, AL) or synthesized according to published procedures (Perly et al., 1984). Cholesterol was provided by Fluka (St. Quentin-Fallavier, France). Pure lipid membranes were prepared by homogenization of lipids in excess deuterium-depleted water. Several freeze-thaw cycles and shaking in a vortex mixer were performed to ensure sample equilibrium. Cholesterol-containing systems were prepared by cosolubilization of lipids and sterol, solvent evaporation, and hydration as described above.

NMR was performed on a Bruker MSL 200 spectrometer operating at 30.7 MHz for deuterium. The phase-cycled quadrupolar echo sequence (Davis et al., 1976) was used with $\pi/2$ pulse widths of 4 μs and a 30-μs delay between the pulses to form the echo. Recycle delay was 2 s, and 1800 acquisitions were recorded. Signal detection was performed in quadrature on a spectral width of 500 kHz. Temperature was regulated to ± 1°C.

Spectral de-Pakeing was performed according to Bloom et al. (1981) and Sternin et al. (1983), except that we chose to calculate oriented-like spectra for bilayer normals oriented at 90° with respect to the external magnetic field direction.

THEORETICAL BACKGROUND

Quadrupolar interaction

The quadrupolar hamiltonian is known to dominate all internal magnetic interactions in a C-D bond system embedded in an anisotropic medium and can be written to the first order as (Mehring, 1976; Spiess, 1978):

$$\mathscr{H}_Q = C_Q T_{20} \sum_{p=-2}^{2} \mathscr{D}^{(2)}_{p0}(\Omega)\, R^P_{2p}, \tag{1}$$

where C_Q is a constant, T_{20} and R^P_{2p} are the tensorial elements of the spin operator and electric field gradient (EFG), respectively. $\mathscr{D}^{(2)}_{m,n}$ are the second rank Wigner matrix elements allowing transformation from the principal axis system, P, to the laboratory axis system, L, using a set of three Euler angles, $\Omega = \phi, \theta, \psi$. Solving (1) leads to the well known quadrupolar splitting observed in ^{2}H-NMR:

$$\Delta\nu_Q = \frac{3}{2} A_Q \mathscr{D}^{(2)}_{00}(\Omega), \tag{2}$$

where A_Q stands for the static quadrupolar coupling constant (A_Q = 167 kHz for methylene C-D bonds (Burnett and Müller, 1971)). It is considered in (2) that the EFG tensor is axially symmetric (Derbyshire et al., 1969).

In the case of a C-D bond undergoing motional averages as in fluid biomembranes, the quadrupolar splitting reduces to:

$$\langle \Delta\nu_Q \rangle = \frac{3}{2} A_Q \frac{3\cos^2\theta_L - 1}{2} \langle \mathscr{D}^{(2)}_{00}(\Omega) \rangle, \tag{3}$$

where the angular brackets stand for a time and space average and θ_L for the Euler angle allowing transformation from the z axis system (the bilayer normal, Z_N) of the last motion of the hierarchy in time of motions with correlation time short enough to produce motional averaging to the magnetic field direction (z axis of the laboratory axis system).

S^{CD} and S^{CC} bond order parameters

There have been numerous studies reporting that intra- and intermolecular motions are well separated in time. Lipid chain isomerization in the fluid phase was reported to have a correlation time, τ_j of $10^{-11}/10^{-12}$ s, whereas molecular rotation occurs in the nanosecond time scale and molecular wobbling at $10^{-7}/10^{-8}$ s (Meier et al., 1986; Weisz et al., 1992). As early as 1977, Petersen and Chan proposed that S^{CD} could be split into molecular ordering and conformational ordering:

$$\begin{aligned} S^{CD} &= \langle \mathscr{D}^{(2)}_{00}(\Omega) \rangle \\ &= \langle \mathscr{D}^{(2)}_{00}(\Omega_{intra}) \rangle \langle \mathscr{D}^{(2)}_{00}(\Omega_{inter}) \rangle. \end{aligned} \tag{4}$$

In writing (4), it is assumed that intra- and intermolecular motions are independent and possess axial symmetry. It must be mentioned here that conformational exchange between C-C bond rotamers is not, in general, axially symmetric. However, by considering the molecule long axis fast rotation, the axial symmetry is achieved at the intramolecular level ($\langle \mathscr{D}^{(2)}_{00}(\Omega_{intra}) \rangle$). The intermolecular motions such as anisotropic reorientation of the whole molecule in an orienting potential (Petersen and Chan, 1977; Meier et al., 1986; Dufourc et al., 1992) are often termed as molecular and lead to $S_{mol} = \langle \mathscr{D}^{(2)}_{00}(\Omega_{inter}) \rangle$, the molecular order parameter. This will be discussed later. In addition, it must be mentioned that collective motions such as order director fluctuations (Stöhrer et al., 1991) have been neglected in (4). For the sake of completeness, they could be introduced through other Wigner matrix elements.

Intramolecular order parameters

The intramolecular contribution can be described as isomerizations around C-C bonds as shown in Fig. 1. Rotation of angle ψ_{isom} around the C_k-C_{k-1} bond leads to the C_k-D order parameter:

$$S^{CD}_k = S_{mol} \left\langle \sum_{p=-2}^{2} \mathscr{D}^{(2)}_{0,p}(0, \theta_{isom}, \psi_{isom} \pm 120°) \times \mathscr{D}^{(2)}_{p0}(0, \theta_k, 0) \right\rangle, \tag{5}$$

where θ_{isom} and $\psi_{isom} \pm 120°$ account for the isomerization (the reference state being the all-*trans* conformation) and θ_k for the coordinate transformation from the C_k-C_{k-1} bond onto the Z_D axis. $\psi_{isom} + 120°$ stands for the azimuthal angle for one deuteron on a given C-D_2 unit, whereas $\psi_{isom} - 120°$

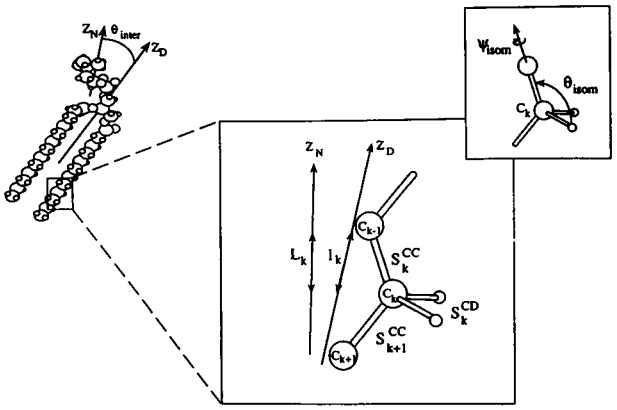

FIGURE 1 Schematic representation of a phospholipid molecule with the axes systems needed for the description of the intermolecular and intramolecular motions. Z_N and Z_D represent the bilayer normal and the diffusion axis of the molecule, S_k^{CD} and S_k^{CC} the C_k-D and the C_k-C_{k-1} bond order parameters, l_k and L_k the average segment length projected onto the diffusion axis and onto the bilayer normal, respectively. θ_{inter} accounts for intermolecular averages, and ψ_{isom} and θ_{isom} account for the intramolecular motions.

stands for the other deuteron on the same unit. It must be emphasized that our description using the Wigner matrices does not require the knowledge of the distribution function of ψ_{isom}, i.e., at this point no conformational model is needed for C-C isomerization.

The same isomerization will lead to S_k^{CC} and S_{k+1}^{CC} order parameters for the C_{k-1}-C_k and C_k-C_{k+1} bonds, respectively:

$$S_k^{CC} = S_{mol}\langle \mathscr{D}_{00}^{(2)}(0, \theta_k, 0)\rangle \tag{6}$$

$$S_{k+1}^{CC} = S_{mol}\sum_{p=-2}^{2}\langle \mathscr{D}_{0p}^{(2)}(0, \theta_{isom}, \psi_{isom})\,\mathscr{D}_{p0}^{(2)}(0, \theta_k, 0)\rangle$$

C-D and C-C bond order parameters can be linked together by (see Appendix):

$$S_k^{CC} + S_{k+1}^{CC} = -2S_k^{CD} \tag{7}$$

when the two deuterons on the same C-D_2 unit are equivalent, and to

$$S_k^{CC} + S_{k+1}^{CC} = -(S_k^{CD}(R) + S_k^{CD}(S)) \tag{8}$$

when they are not (e.g., lipids labeled at the $C_{2'}$ position in the *sn*-2 chain (Seelig and Niederberger, 1974; Engel and Cowburn, 1981; Dufourc et al., 1983). Equations 7 and 8 indicate that C-C order parameters can be obtained from measurement of C-D order parameters, i.e., quadrupolar splittings. Another interesting case is that of the CD_3 terminal on a fatty acyl chain. It is well known that there is fast axial rotation of the methyl group around the C_{n-1}-C_n bond; hence, the S_n^{CC} order parameter can be written as:

$$S_n^{CC} = \frac{S_n^{CD}}{(3\cos^2(111°) - 1)/2}, \tag{9}$$

where the angle between the C_n-D bond and the C_{n-1}-C_n was taken as 111° instead of 109.5°; it indeed appears from force field calculations (Allinger et al., 1989) that this value depicts more realistically the geometry of the methyl terminal. Knowledge of this order parameter allows, with the help of (7) and of the S^{CD} profile, calculation of the S^{CC} profile along the acyl chain (vide infra).

Intermolecular order parameter, S_{mol}

This order parameter accounts for intermolecular motional averages (see Fig. 1) and may be defined as:

$$S_{mol} = \frac{3\langle\cos^2\theta_{inter}\rangle - 1}{2}, \tag{10}$$

with

$$\langle\cos^2\theta_{inter}\rangle = \frac{\int_0^{\theta_{max}}\cos^2\theta_{inter}\sin\theta_{inter}\,d\theta_{inter}}{\int_0^{\theta_{max}}\sin\theta_{inter}\,d\theta_{inter}} \tag{11}$$

In writing (11), it is assumed that the molecule is confined in a cone of semi-angle θ_{max} (Petersen and Chan, 1977; Oldfield et al., 1978a, b). This order parameter is easily measurable in rigid molecules (no conformational disorder) labeled with deuterium. The observable S^{CD} is then directly proportional to S_{mol}. This has been used successfully in studies with cholesterol or cyclopropane groups in fatty acyl chains (Dufourc et al., 1983, 1984; Léonard and Dufourc, 1991). In flexible molecules such as saturated fatty acyl chains, the observable S^{CD} is proportional to both S_{mol} and conformational order (4) (Petersen and Chan, 1977; Oldfield et al., 1978a, b). It must be pointed out here that one could have chosen a more complex distribution function for (11) as proposed, for instance, in Meier et al. (1986) or Weisz et al. (1992). Although (11) may appear to be a rough description of molecular wobbling in an orienting potential, it has the advantage over more complex expressions in that it allows analytical calculation of the contribution of chain wobbling to the average length, without including a new parameter (such as a Maier-Saupe potential), which often is difficult to determine. We believe that for purposes of chain length determination, this approximation does not lead to much larger inaccuracies than the other approaches.

Hydrocarbon chain length

Schindler and Seelig (1975) have proposed using the S^{CD} profile to calculate the average acyl chain length of a lipid and, hence, the bilayer thickness. Their model is based on fast exchange between three conformers for each methylene unit. An angle β is used to denote the orientation of a "segment" that is in fact the normal to the plane, $\vec{n}_k$, spanned by the two C-D bonds with respect to the molecule long axis. In addition, they assumed that $S_{mol} = 1$, which implies a colinearity between Z_D and the bilayer normal, Z_N. This need not to be the case if intermolecular motions are present. These authors only consider three "segment" orientations, namely, $\beta = 0°$, 60°, and 90°. However, if one wishes to calculate the average conformation of a C-C bond, one needs to take into account, in the most general case (Flory, 1969; Meier et al., 1986), four rotamers as sketched in Fig. 2.

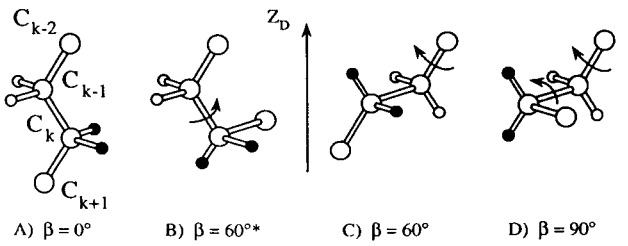

FIGURE 2 The four possible conformers that may occur at a C_k-D_2 unit. The angle β describes the orientation of the normal to the C_k-D_2 plane with respect to the diffusion axis.

Case A occurs, for instance, for the all-*trans* configuration; $\vec{n}_k$ is then oriented at 0° and $S_k^{CD} = -0.5\,S_{mol}$ and $S_k^{CC} = S_{k+1}^{CC} = 0.5\,S_{mol}$. Note that the C_{k-1}-C_k and C_k-C_{k+1} bonds are both oriented at 35.5°. Case B appears when a *gauche* +, (g+), or *gauche* −, (g−), isomerization takes place around the C_{k-1}-C_k bond. $\vec{n}_k$ is then oriented at $\beta = 60°$, and one sees that the C_k-C_{k+1} bond is oriented at 90° with respect to the diffusion axis Z_D, $S_k^{CC} = 0.5\,S_{mol}$ and $S_{k+1}^{CC} = -0.5\,S_{mol}$. In case C, isomerization takes place around the C_{k-2}-C_{k-1} bond, $\vec{n}_k$ is also oriented at $\beta = 60°$, but note that the C_k-C_{k+1} bond is now oriented at 35.5°, $S_k^{CC} = -0.5\,S_{mol}$, and $S_{k+1}^{CC} = 0.5\,S_{mol}$. The β angle for case B will be denoted 60*. In these two cases (B and C), the order parameter S_k^{CD} is equal to 0, because of the equivalence of the two deuterons. Case D may be obtained for a sequence of g+g+ bonds (or g−g−), $\vec{n}_k$ is then oriented at 90°, and $S_k^{CD} = +0.5\,S_{mol}$, and both C-C bonds before and after C_k are oriented at 90°, $S_k^{CC} = S_{k+1}^{CC} = -0.5\,S_{mol}$. By considering that these four conformers are in rapid exchange in the NMR time scale, one can now calculate the S_k^{CD}, S_k^{CC}, and S_{k+1}^{CC} order parameters in terms of the probabilities P_β^k of each of the conformers.

$$\begin{aligned} 2S_k^{CD} &= S_{mol}\,(-P_0^k + P_{90}^k) \\ 2S_k^{CC} &= S_{mol}\,(P_0^k - P_{90}^k + P_{60^*}^k - P_{60}^k) \\ 2S_{k+1}^{CC} &= S_{mol}\,(P_0^k - P_{90}^k - P_{60^*}^k + P_{60}^k), \end{aligned} \tag{12}$$

with $P_0^k + P_{90}^k + P_{60}^k + P_{60^*}^k = 1$. It must be mentioned here that the S^{CC} part of (12) could not be calculated according to Seelig's definition of "segment" conformation. What can be calculated with his approach is the order parameter of what is called a "segment," i.e., the normal to a D-C_k-D plane, in terms of probabilities P_0^k, P_{90}^k, and P_{60}^k of his three conformers (Eq. 9 in Schindler and Seelig, 1975). It is interesting to remark that addition of the two last lines of (12) leads to (7). As already indicated above, the latter has been derived in a general way, without any need of conformer definition; this reinforces the choice of four conformers to describe C-C bond isomerization.

Eq. (7) may be rewritten in terms of (12) as:

$$S_k^{CC} + S_{k+1}^{CC} = S_{mol}\,(P_0^k - P_{90}^k). \tag{13}$$

The average length $\langle L_{k+1}\rangle$ with respect to the Z_N axis of an aliphatic chain segment is obtained by considering two successive projections: the first one from the C_k-C_{k+1} bond onto the Z_D axis (accounting for intramolecular motions) and the second one from the Z_D axis to the Z_N axis (accounting for intermolecular motions, see Fig. 1). We must be reminded here that this is allowed by consideration of independent intra- and intermolecular motions (vide supra). The intramolecular contribution to a chain segment is then given by (see Figs. 1 and 2):

$$\langle l_{k+1}\rangle = l_0\,(P_0^k + P_{60}^k), \tag{14}$$

where $l_0 = 1.54\cos(35.5°)$ Å. Introducing the S^{CC} order parameter into (14), it becomes:

$$\langle l_{k+1}\rangle = 1.25\left\{\frac{1}{2} + \frac{S_{k+1}^{CC}}{S_{mol}}\right\}. \tag{15}$$

The average length $\langle L_{k+1}\rangle$ is obtained by considering the intermolecular contribution:

$$\langle L_{k+1}\rangle = \langle\cos\theta_{inter}\rangle\,\langle l_{k+1}\rangle, \tag{16}$$

where

$$\begin{aligned} \langle\cos\theta_{inter}\rangle &= \frac{\int \cos\theta_{inter}\sin\theta_{inter}\,d\theta_{inter}}{\int \sin\theta_{inter}\,d\theta_{inter}} \\ &= \frac{1}{4}(1 + \sqrt{1 + 8\,S_{mol}}). \end{aligned} \tag{17}$$

The average chain length, $\langle L_{chain}\rangle$, is obtained by adding each average aliphatic chain segment:

$$\langle L_{chain}\rangle = \frac{(1 + \sqrt{1 + 8\,S_{mol}})}{4} \times \left[\langle l_{C_n-H}\rangle + 1.25\sum_{k=2}^{n}\left(\frac{1}{2} + \frac{S_k^{CC}}{S_{mol}}\right)\right], \tag{18}$$

where the contribution of the C-H bond on the methyl terminus is estimated to be $\langle l_{C_n-H}\rangle = \cos(35.5°)\,1.09$ Å = 0.81 Å. It must be noted that $\langle L_{chain}\rangle$ is a measure from carbon-1 to carbon n. Equation 18 may be written in terms of S^{CD} order parameters for chains with even number of carbons:

$$\langle L_{chain}\rangle = \frac{(1 + \sqrt{1 + 8\,S_{mol}})}{4} \times \left[\langle l_{C_n-H}\rangle + 1.25\left(\frac{1}{2} + \frac{S_n^{CC}}{S_{mol}} + \sum_{m=1}^{n/2-1}\left(1 + \frac{2\,S_{2m}^{CD}}{S_{mol}}\right)\right)\right], \tag{19}$$

where S_n^{CC} is obtained by (9). For chains with an odd number of carbons, we obtain:

$$\langle L_{chain}\rangle = \frac{(1 + \sqrt{1 + 8\,S_{mol}})}{4} \times \left[\langle l_{C_n-H}\rangle + 1.25\sum_{m=1}^{n/2-1}\left(1 + \frac{2\,S_{2m}^{CD}}{S_{mol}}\right)\right]. \tag{20}$$

It is interesting to note that (19) and (20) only need to take into account even S^{CD} order parameters.

Kink and jog propagation

As proposed by Seelig and co-workers (Schindler and Seelig, 1975; Seelig, 1977), the chain flexibility may alternatively be described by the propagation of conformational defects from the glycerol backbone to the bilayer center. Among all chain defects, the above authors found that *kinks* ($g^+ t g^-$ or $g^- t g^+$ sequences) or *jogs* ($g^+ t_{2i+1} g^-$, 2i+1 = number of consecutive *trans* conformations) are the most probable because they leave the chains essentially parallel to each other in the bilayer. According to this model, this corresponds to the occurrence of cases A, B, and C only (Fig. 2). The S^{CD} and S^{CC} order parameters can then be written as:

$$2S_k^{CD} = S_{mol} P_0^k$$
$$2S_k^{CC} = S_{mol}(1 - 2P_{60}^k) \quad (21)$$
$$2S_{k+1}^{CC} = S_{mol}(1 - 2P_{60*}^k).$$

Knowledge of S^{CD}, S^{CC}, and S_{mol} order parameters, therefore, allows calculation of the P_0, P_{60}, and P_{60*} for each carbon atom along the fatty acyl chain.

RESULTS

S^{CD} order parameters

Solid state ^{2}H-NMR experiments have been performed on several biomembranes made of phosphatidylcholine-water dispersions with and without cholesterol. The *sn*-2 chain was either perdeuterated or selectively labeled. Experiments were performed as a function of temperature on DLPC-, DMPC- (without and with 30% cholesterol), and DPPC-water dispersions. Fig. 3 shows a typical deuterium powder spectrum for the DLPC system in the L_α phase together with the "de-Paked" analog on which the quadrupolar splittings are easily measurable. Assignment of labeled positions was performed on the basis of previously published data on selectively deuterated systems (Seelig and Seelig, 1974; Seelig and Niederberger, 1974; Oldfield et al., 1978b).

S^{CD} order parameters were then calculated and reported in Tables 1–3. Table 1 collects data on the DMPC and DMPC + 30 mol% cholesterol (Dufourc et al., 1984). In the case of the pure system, we report data on specifically labeled lipids (Oldfield et al., 1978b). S^{CD} values for missing positions in Oldfield's data (labeled with a plus sign in Table 1) were obtained by application of a temperature correction to well resolved positions (i.e., positions out of the "plateau") in the same system in which the *sn*-2 chain was perdeuterated (data not shown). For positions within the "plateau," a linear interpolation was applied. In this table S_{mol} values for DMPC as a function of temperature are also reported (Dufourc et al., 1992) and for DMPC-cholesterol at 25°C (Dufourc et al., 1984; Léonard and Dufourc, 1991). In Table 2, we collect DLPC data, and in Table 3, we report on DPPC. For the latter,

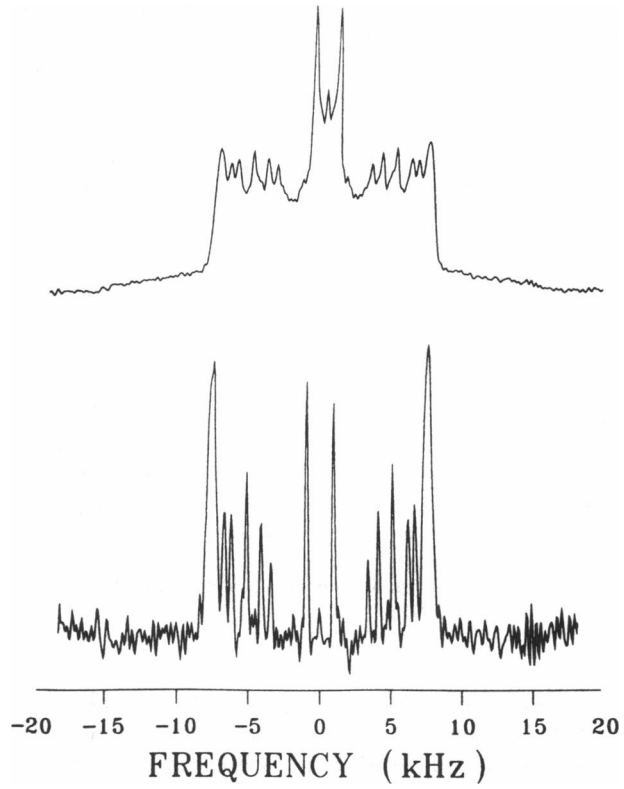

FIGURE 3 Typical ^{2}H-NMR powder spectrum of [*sn*-2 $^2H_{23}$]DLPC in excess water (*top*) and the corresponding de-Paked spectrum (*bottom*). De-Paking was performed such that the bilayer normal is oriented at 90° with respect to the magnetic field (see text for details).

resonance assignment was performed with the help of DPPC selectively labeled in carbon-3 of the *sn*-2 chain and other selectively deuterated positions from Seelig (1977) and Engel and Cowburn (1981). DLPC data for labeled position 2 could not be measured accurately and are not reported in Table 2.

All biomembrane systems exhibit the well known behavior for the S^{CD} parameters, i.e.: 1) the inequivalence of the two deuterons on the carbon-2 position (for DMPC and DPPC); 2) the length of the "plateau" of order parameters, i.e., S^{CD} is quasi constant from C_3 to C_{8-11}, decreases with increasing temperature; 3) a marked decrease in order when the temperature increases (except for C_2); and 4) a dramatic increase in S^{CD}, for all labeled positions, in the presence of cholesterol, as already documented (Oldfield et al., 1978b; Dufourc et al., 1984; Léonard and Dufourc, 1991).

S^{CC} order parameters

From (9) one may calculate S^{CC} for the terminal C-C bond, S_n^{CC}, where n is the total number of carbon atoms in the chain. It must be mentioned here that S_n^{CD} is considered as negative. Indeed, at low temperatures ($T \ll T_c$) where there is no longer bond isomerizations in the chain, the rotation around the C_{n-1}-C_n bond is still very fast and leads a negative S^{CD} value for geometrical reasons (C_n-D bonds at 111° with respect to

TABLE 1 S^{CD} and S_{mol} order parameters as a function of temperature and labeled carbon position on the sn-2 chain, for DMPC and DMPC + 30% cholesterol

T§ / k‡	23	30	35	40	45	50	55	60	*
2S¶	0.096	0.094	0.094	0.094	0.093	0.093	0.093	0.093	0.130
2R¶	−0.162	−0.147	−0.141	−0.137	−0.133	−0.130	−0.128	−0.126	−0.258
3	−0.236	−0.221	−0.212	−0.204	−0.198	−0.193	−0.190	−0.189	−0.374
4	−0.248	−0.232	−0.220	−0.212	−0.204	−0.196	−0.190	−0.185	−0.433
+5	−0.250	−0.232	−0.220	−0.212	−0.204	−0.196	−0.190	−0.185	−0.433
6	−0.252	−0.234	−0.222	−0.212	−0.204	−0.196	−0.190	−0.185	−0.433
7	−0.250	−0.230	−0.216	−0.206	−0.198	−0.192	−0.185	−0.179	−0.433
8	−0.244	−0.224	−0.210	−0.199	−0.190	−0.181	−0.173	−0.165	−0.433
+9	−0.230	−0.208	−0.198	−0.184	−0.174	−0.168	−0.160	−0.154	−0.433
10	−0.213	−0.190	−0.176	−0.164	−0.156	−0.146	−0.140	−0.134	−0.433
+11	−0.194	−0.177	−0.161	−0.154	−0.148	−0.132	−0.126	−0.122	−0.398
12	−0.170	−0.150	−0.139	−0.129	−0.121	−0.114	−0.107	−0.101	−0.354
+13	−0.141	−0.121	−0.111	−0.102	−0.096	−0.089	−0.086	−0.078	−0.294
14	−0.037	−0.031	−0.027	−0.025	−0.023	−0.019	−0.018	−0.018	−0.078
S_{mol}†	0.724	0.718	0.711	0.704	0.695	0.685	0.677	0.667	0.800

*DMPC + 30% cholesterol at 25°C from Dufourc et al. (1984).
‡Labeled carbon position on the *sn*-2 chain. The + indicates that corresponding positions were interpolated in Olfield's data (see text).
§Temperature in °C.
¶2R = *pro*-R and 2S = *pro*-S positions according to Engel and Cowburn (1981).
†From Dufourc et al. (1992).

the C_3 symmetry axis). Because the quadrupolar splitting for the methyl terminal never goes through zero on going to the $L_{\beta'}$ phase (Léonard and Dufourc, 1991), one may then reasonably assume a negative value in the L_α phase. Using (7) it is now easy to calculate each S_k^{CC} on going up in the acyl chain. Here, one has to mention that S_k^{CD} are also negative (12). For the two nonequivalent deuterons in position C_2, one may use x-ray data (Hauser et al., 1981) to find that $S_2^{CD}(R)$ is negative and $S_2^{CD}(S)$ is positive (Engel and Cowburn, 1981). The above approach has been applied to all data in Tables 1–3, allowing us to plot the variation of S_k^{CC} against k (Fig. 4). In this figure, for the sake of clarity, only two representative temperatures ($T \sim T_c$ and $T \gg T_C$) have been plotted for all biomembranes. S_2^{CC} has been plotted separately in Fig. 6. A general observation may be made for all systems: not surprisingly, one obtains a S^{CC} profile (from C_3 to C_n) that in some way parallels that of S^{CD}. However, for all biomembranes the S^{CC} order parameters exhibit a marked odd-even behavior, i.e., $S_4^{CC} < S_5^{CC}$; $S_6^{CC} < S_7^{CC}$, etc., especially for $T \gg T_C$. The amplitude of this odd-even effect clearly increases with temperature. To monitor this effect, we calculated the temperature variation of the difference, Δ, between consecutive odd and even order parameters:

$$\Delta = \frac{1}{N-3}\sum_{k=3}^{N}\left|S_k^{CC} - S_{k+1}^{CC}\right|. \qquad (22)$$

The summation is over the *plateau* order parameters, where $N - 3$ is the number of C-C order parameters taken into account. For DLPC, DMPC, and DPPC, the *plateau* was defined as ending at positions $N = 8$, 10, and 12, respectively. In Fig. 5 we plotted Δ values for DPPC, DMPC, and DLPC as a function of temperature. Interestingly, the Δ value increases dramatically with temperature for all systems. However, it seems that there is a lag for DLPC, i.e., Δ is small and almost constant for some 20°C after the phase transition temperature and then increases for $T \gg T_C$. For DMPC and DLPC, Δ appears to level off at $T - T_C \geq 30$°C.

S_2^{CC} are plotted as a function of temperature for DMPC and DPPC (Fig. 6). One notes that S_2^{CC} is negative and becomes

TABLE 2 S^{CD} order parameters as a function of temperature and labeled carbon position on the DLPC sn-2 chain

T‡ / k*	5	10	15	20	25	35	45
3	−0.270	−0.238	−0.223	−0.213	−0.202	−0.188	−0.178
4	−0.270	−0.238	−0.233	−0.213	−0.202	−0.188	−0.178
5	−0.270	−0.238	−0.223	−0.213	−0.202	−0.188	−0.178
6	−0.270	−0.238	−0.233	−0.213	−0.202	−0.188	−0.178
7	−0.270	−0.238	−0.223	−0.213	−0.202	−0.188	−0.178
8	−0.270	−0.238	−0.218	−0.205	−0.189	−0.170	−0.157
9	−0.258	−0.214	−0.192	−0.175	−0.164	−0.148	−0.134
10	−0.235	−0.185	−0.160	−0.143	−0.134	−0.120	−0.106
11	−0.185	−0.142	−0.121	−0.111	−0.101	−0.089	−0.078
12	−0.047	−0.033	−0.029	−0.026	−0.023	−0.019	−0.016

*Labeled carbon position on the *sn*-2 chain.
‡Temperature in °C.

TABLE 3 S^{CD} order parameters as a function of temperature and labeled carbon position on the DPPC sn-2 chain

$T^{\ddagger}$ / k^{*}	41	44	50	57	65	73
2S[§]	0.100	0.099	0.097	0.100	0.096	0.100
2R[§]	−0.153	−0.144	−0.142	−0.133	−0.128	−0.122
3	−0.218	−0.212	−0.203	−0.193	−0.184	−0.178
4	−0.241	−0.230	−0.217	−0.198	−0.187	−0.181
5	−0.241	−0.230	−0.217	−0.198	−0.187	−0.181
6	−0.241	−0.230	−0.217	−0.198	−0.187	−0.181
7	−0.241	−0.230	−0.217	−0.198	−0.187	−0.175
8	−0.241	−0.229	−0.217	−0.193	−0.187	−0.170
9	−0.232	−0.216	−0.205	−0.186	−0.178	−0.160
10	−0.227	−0.212	−0.190	−0.174	−0.160	−0.142
11	−0.217	−0.201	−0.179	−0.162	−0.146	−0.129
12	−0.200	−0.181	−0.162	−0.145	−0.128	−0.115
13	−0.185	−0.166	−0.147	−0.133	−0.118	−0.110
14	−0.155	−0.137	−0.122	−0.107	−0.096	−0.086
15	−0.123	−0.110	−0.098	−0.087	−0.076	−0.069
16	−0.033	−0.029	−0.026	−0.023	−0.020	−0.018

*Labeled carbon position on the *sn*-2 chain.
‡Temperature in °C.
§2R = *pro*-R and 2S = *pro*-S positions according to Engel and Coburn (1981).

more negative as the temperature increases. The slope appears to be steeper for DPPC than for DMPC. Unfortunately, no reliable data could be obtained for DLPC. A value for DMPC in the presence of cholesterol at 25°C has also been plotted and corresponds to what is observed for DMPC alone at 50–55°C.

Propagation of *gauche* defects

As introduced in the Theory, the propagation of *gauche* defects along the acyl chain with a minimal increase in chain volume may be modeled by *kink* and *jog* conformations. In the frame of this model, one may calculate the P_0^k, P_{60}^k, and P_{60*}^k probabilities as a function of labeled carbon position, temperature, or cholesterol content. For such a calculation, (21) has been used with the S_{mol} value obtained on DMPC systems from Léonard and Dufourc (1991) and Dufourc et al. (1992) (Fig. 7).

For the pure lipid system and near T_C, the P_0 probability dominates all other probabilities for all chain positions (Fig. 7 *a*). Near the methyl terminal, one notes, however, that P_0^k, P_{60}^k, and P_{60*}^k are almost equal. Increasing the temperature (Fig. 7 *b*) leads to a decrease in P_0^k and a concomitant increase in P_{60}^k and P_{60*}^k, reflecting the temperature-driven increase in *gauche* defects. This is of particular importance near the bilayer center (Fig. 7 *b*), where P_{60}^k and P_{60*}^k are greater than P_0^k. The effect of cholesterol on theses profiles is striking at 25°C: the P_0^k probability is very close to 1 for almost all positions, and there are only a few *gauche* conformers detected near the chain end. For higher temperatures, the main features of the cholesterol-containing system are conserved with however a slight decrease of P_0^k and a concomitant increase of P_{60}^k and P_{60*}^k (data not shown).

It is interesting to determine the number of *gauche* defects per chain, g_{chain}. This can easily be performed in our study by summing over k all P_{60*}^k probabilities. Results are reported in Fig. 8 for all systems. It must be mentioned that in the case of DLPC and DPPC we have no information on S_{mol}, and it has been considered that $S_{mol} = 1$. For comparison, we have reported in this figure values for DMPC with S_{mol} taken from Table 1 and with $S_{mol} = 1$. It appears clearly that taking $S_{mol} = 1$ leads to an overestimation of g_{chain} by ~1 *gauche*, independent of the temperature. Assuming that the same overestimation is made for the other systems, g_{chain} would vary within the temperature range of the study from 1.9 to 3.2 for DLPC and from 3.5 to 4.4 for DPPC. Interestingly, the number of *gauche* defects increases with chain length and temperature with respect to T_C. The presence of cholesterol leads to a marked decrease of g_{chain} from 2.8 to 0.8 at 25°C (data not shown).

Average chain length

The average length of the *sn*-2 acyl chain, $\langle L_{chain} \rangle$, has been calculated according to (18) and plotted on Fig. 9 in the case of DMPC. Molecular order parameter values for corresponding temperatures have been taken from Dufourc et al. (1992). Because S_{mol} values are not always known, we also plotted on this graph $\langle L_{chain} \rangle$ by taking $S_{mol} = 1$. Not surprisingly, both curves are very close when the system is near to the phase transition and depart from each other when increasing the temperature. At 60°C, taking $S_{mol} = 1$ leads to an overestimation of the chain length by 0.5 Å, i.e., about 4%. The presence of cholesterol markedly increases the chain length by 3 Å at 25°C (data not shown), in agreement with previous findings (Léonard and Dufourc, 1991). We also plotted the thermal dependence of the DLPC and DPPC *sn*-2 chains on the same graph (with $S_{mol} = 1$). It is interesting to note that for all systems the chain length rapidly decreases when increasing the temperature immediately after T_c and levels off for $T \gg T_c$.

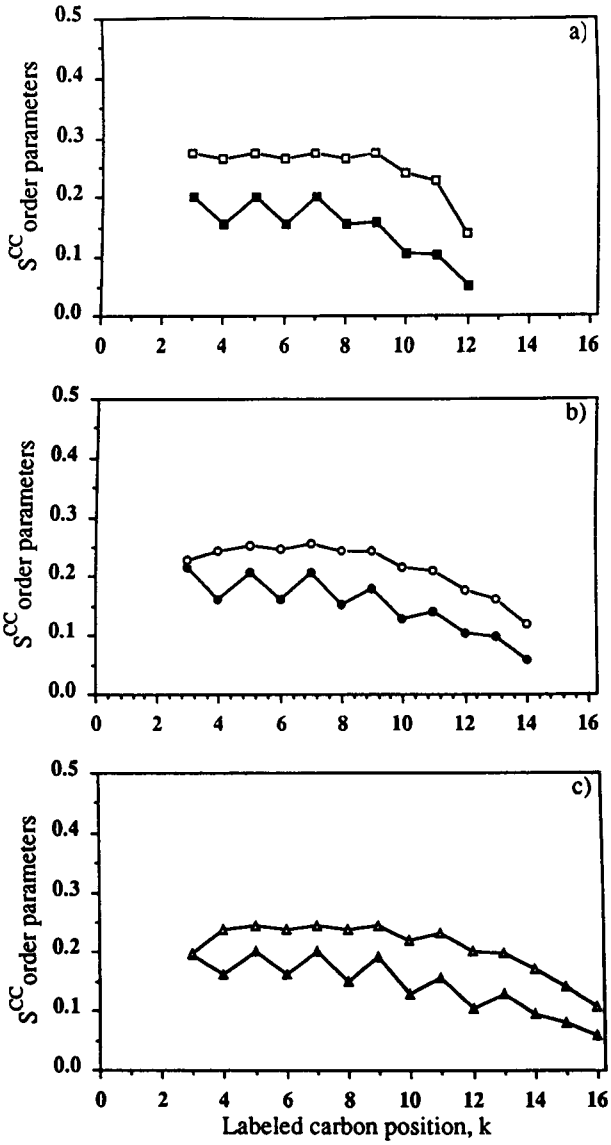

FIGURE 4 S^{CC} order parameters as a function of the labeled carbon position, k. (*a*) DLPC at 5°C (□) and 45°C (■); (*b*) DMPC at 23°C (○) and 60°C (●); (*c*) DPPC at 41°C (△) and 73°C (▲). Accuracy is within symbol size.

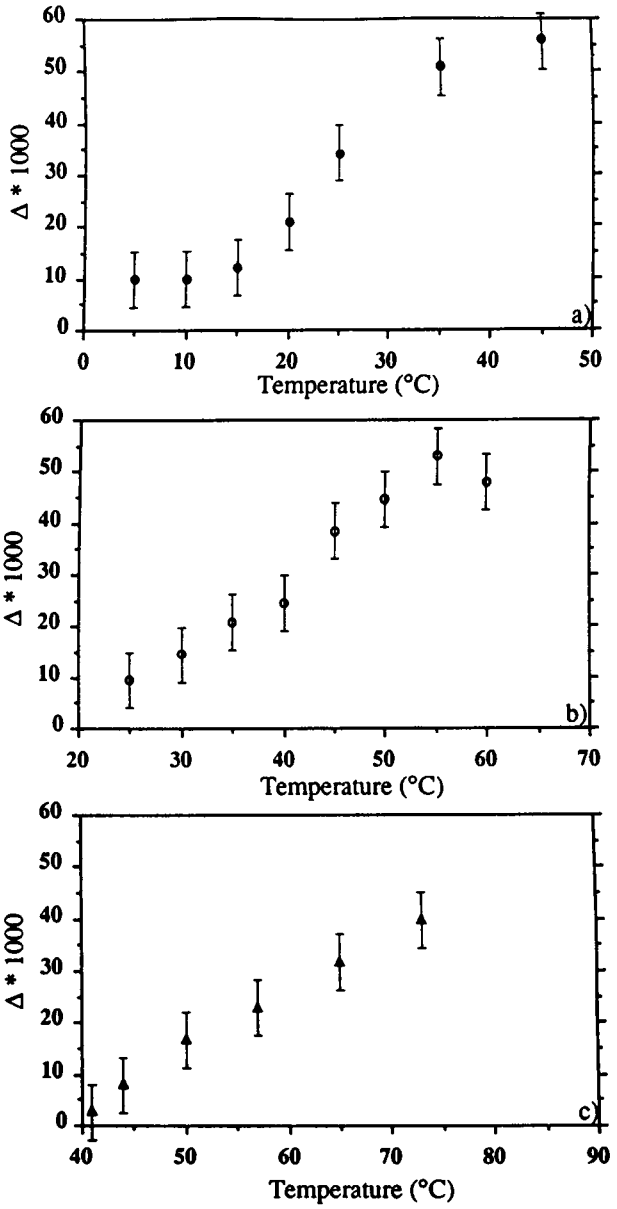

FIGURE 5 Amplitude of the odd-even effect, Δ (see text for definition), as a function of temperature. (*a*) DLPC; (*b*) DMPC; (*c*) DPPC.

DISCUSSION

Calculation of S^{CC} order parameters brings new and valuable information that did not appear from S^{CD} measurements. They can be summarized as follows. 1) There are marked differences between odd and even S^{CC} order parameters, and they increase when increasing temperature above T_C, for all biomembrane systems. 2) Calculation of S_2^{CC} from nonequivalent deuterons on carbon-2 affords determination of the geometry of the beginning of the chain attached to the glycerol backbone. 3) The fatty acyl chain length can be calculated from carbon-1 to the methyl terminal, n, with the knowledge of the C-C bond length and the S^{CC} profile. 4) It is possible to determine the probabilities of each conformer at a given carbon position as well as the number of *gauche* defects (*kink* and *jog*) per chain. In what follows, we will discuss in detail the above points and, finally, compare our method with that proposed by Schindler and Seelig (1975).

The odd-even effect

Marked differences between odd and even S^{CC} order parameters, the so called odd-even effect, become very important at high temperatures relative to T_C and reflect in some way the conformational behavior of the chain end, because the calculation is performed from the methyl terminal. The magnitude of the effect is much more important with S^{CC} order parameters compared with S^{CD}, especially at $T \gg T_C$. The Δ

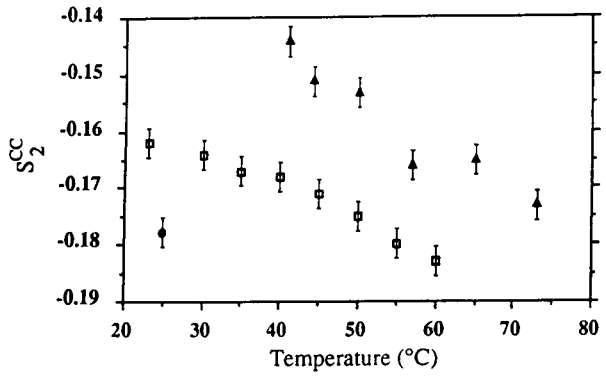

FIGURE 6 Temperature variation of the C_1-C_2 bond order parameter, S_2^{CC}, for DMPC (□) and DPPC (▲). (●) DMPC + 30 mol% cholesterol.

parameter we defined to quantitate this effect markedly increases from ~10% close to T_C to ~50% at $T - T_C \approx 40°C$, where it seems to reach a plateau for DLPC and DMPC. Until now, we unfortunately have no model to account for the increase of the odd-even effect as the temperature increases. Interestingly, the thermal behavior of Δ seems to be general for all biomembrane systems investigated.

Orientation of the C_1-C_2 bond

Our model affords the calculation of the C_1-C_2 bond order parameter (8), i.e., it leads to the average orientation of the O=C—C_2 group linked to the glycerol backbone. Interestingly, for the DMPC system, S_2^{CC} is negative (Fig. 6) and decreases from −0.16 at 25°C to −0.18 at 60°C. This suggests that the C_1-C_2 bond is not totally anchored and that *trans-gauche* isomerizations take place in this region of the aliphatic chain. The negative sign clearly indicates the preference for the bond to be oriented near 90° with respect to the Z_D axis, which is in good agreement with x-ray observations (Hauser et al., 1981). Interestingly, S_2^{CC}(DPPC) is less negative than S_2^{CC}(DMPC), which indicates that the average bond orientation is closer to 90° for DMPC than for DPPC. This is in accordance with the general concept of increase in chain packing (increase in Van der Waals interactions) when the chain length increases. Chains of DPPC would be more extended than in DMPC, which is reflected by a tendency for the C_1-C_2 bond to be less parallel to the bilayer plane. Temperature increase leads to a decrease in chain constraints and frees the configurations at C_2, which is reflected by a more negative S_2^{CC} value, i.e., a propensity for the C_1-C_2 bond to become more parallel to the bilayer surface. Interestingly, the effect of temperature is more important on DPPC than on DMPC (steeper slope), which is again in agreement with the fact that longer acyl chains are more constrained than shorter ones and, therefore, have more conformational freedom to recover when the temperature is increased.

The presence of 30 mol% cholesterol in DMPC at 25°C leads to a decrease from −0.165 to −0.180, indicating that, on average, the C_1-C_2 bond becomes more parallel to the bilayer surface. This is somewhat surprising in view of the above argumentation because it is known that cholesterol increases the Van der Waals interactions in the bilayer core. One would then favor a situation where the C_1-C_2 bond is stretched further toward the bilayer core as for DPPC. This is clearly not the case and suggests that cholesterol is sufficiently embedded in the membrane to exert Van der Waals interactions in the core and sterical constraints in the C_1-C_2 segment of the *sn*-2 chain to favor its orientation more parallel to the surface.

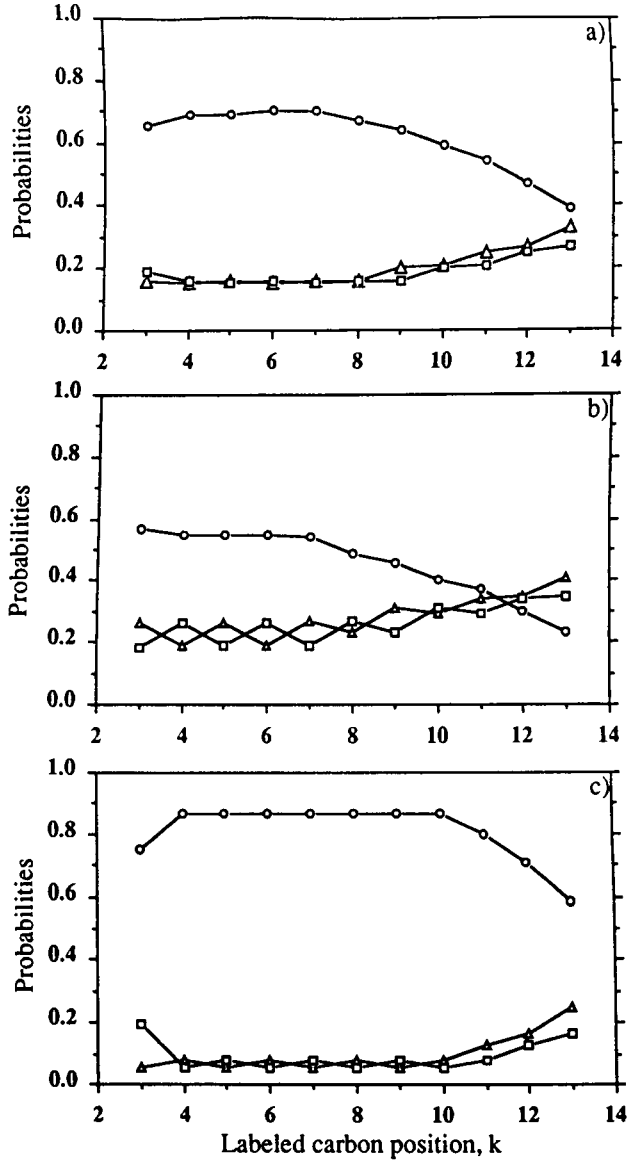

FIGURE 7 Probabilities, P_0, P_{60}, and $P_{60'}$ of the configurations A (○), B (□), and C (△) (see Fig. 2), respectively, as a function of labeled carbon position. (*a*) DMPC at 23°C; (*b*) DMPC at 60°C; (*c*) DMPC + 30 mol% cholesterol at 25°C. Accuracy is within symbol size.

Gauche defects

As demonstrated in Theory, the *kink* and *jog* model together with the knowledge of the S^{CC} order parameters brings a detailed mapping of segment conformations in the acyl

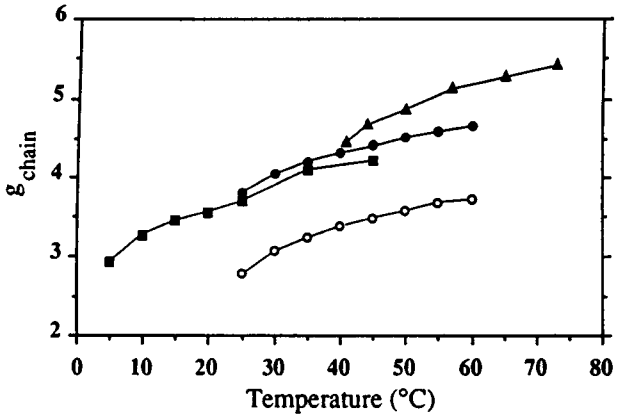

FIGURE 8 Thermal variation of the total number of *gauche* defects per chain, $g_{chain} = \sum_k P^k_{60°}$. (○) DMPC, S_{mol} taken from Table 1; (●) DMPC, $S_{mol} = 1$; (■) DLPC, $S_{mol} = 1$; (▲) DPPC, $S_{mol} = 1$. Accuracy is within symbol size.

chain. The probability P^k_0 (Fig. 7) describes the degree of order in the aliphatic chain. It decreases when going toward the chain end as the disorder increases. A P^k_0 value of 0.7 is observed in the *plateau* region, at 25°C. This value is in remarkable agreement with that obtained by Kothe and coworkers (Mayer et al., 1990) from spin-lattice relaxation measurements on [6′,6′-$^2H_2$]DMPC. The probabilities P^k_{60} and $P^k_{60°}$, which reveal the defects along the chain, slowly increase on going toward the chain end. For the DMPC system at 23°C, P^k_{60} and $P^k_{60°}$ are always lower than P^k_0, whereas at 60°C, these probabilities are greater than P^k_0 for positions 12 and 13. This clearly indicates the increase of *gauche* defects, preferentially in the bilayer core, with temperature. Note also that the condensing effect of cholesterol is nicely reflected by a tremendous increase in segment order as reflected by a value of 0.90 for the plateau positions. Again, this agrees well with the data on [6′,6′-$^2H_2$]DMPC + 40% cholesterol at 35°C, where a n_1 value (our P^k_0) of 0.98 (Mayer et al., 1990) was reported. It is interesting at this point of the discussion to compare our data with those obtained by infrared spectroscopy on DPPC and DPPC/cholesterol mixtures (Casal and McElhaney, 1990; Mendelsohn et al., 1991; Senak et al., 1992). At 50°C, in the absence of cholesterol, these authors found that the amount of *gauche* conformations at positions 4, 6, 10, 13, 14, and 15 were 0.21, 0.30, 0.20, 0.17, 0.40, and 0.40, respectively. Addition of 33 mol% cholesterol reduced the corresponding values to 0.04, 0.04, 0.13, and 0.11; no values were determined for positions 14 and 15. These values are in general agreement with the $P^k_{60°}$ we found, which represents the amount of *gauche* conformers at position k. It must be noted that we were only able to determine accurately the conformer probabilities in the case of DMPC and DMPC/cholesterol because S_{mol} values were available. By taking $S_{mol} = 1$, one overestimates the *gauche* conformers at a given carbon position by 30–60%. The total amount of *gauche* conformers per chain, g_{chain} (Fig. 8), appears to be overestimated by 1 unit when $S_{mol} = 1$. Mendelsohn and co-workers also reported g_{chain} values of 3.9 and 1.2 for DPPC and DPPC + 33% cholesterol at 50°C. Correcting for the overestimation due to $S_{mol} = 1$ in Fig. 8 leads to a g_{chain} of 3.8 for DPPC at 50°C, which is in remarkable agreement with the IR data. The above authors reported a factor 3.2 decrease for g_{chain} due to the presence of cholesterol in DPPC. In the case of DMPC, our data at 25°C indicate a factor 3.7 decrease in the presence of cholesterol. The nice agreement between NMR and infrared data indicates that the *kink* and *jog* model is a fairly good description of the propagation of defects along the acyl chain.

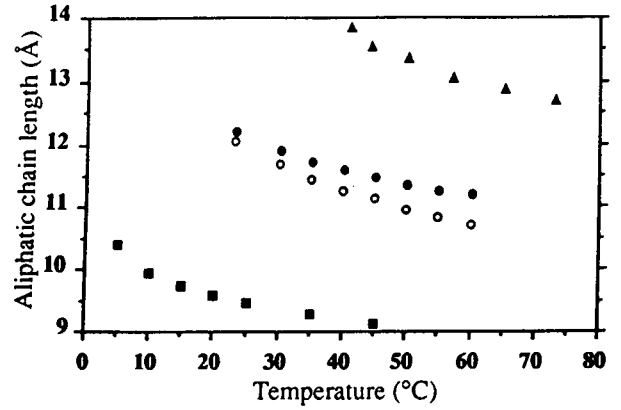

FIGURE 9 Aliphatic chain length (calculated from Eq. 18 of text) as a function of temperature. (○) DMPC, S_{mol} taken from Table 1; (●) DMPC, $S_{mol} = 1$; (■) DLPC, $S_{mol} = 1$; (▲) DPPC, $S_{mol} = 1$. Accuracy is within symbol size.

Chain length

Here we have to mention that the proper length determination could only be made in the case of DMPC where S_{mol} values are known. One sees, nonetheless, that near the phase transition the overestimation due to the calculation with $S_{mol} = 1$ stays within the experimental error and is only of 4% at high temperature with respect to T_C. The thermal behavior of the aliphatic chain length appears to be similar for all biomembrane systems: there is a decrease of ~1.5 Å on going from temperatures near T_C to temperatures some 30–40°C above. One must mention here that in the case of DLPC the C_1-C_2 segment was not taken into account because we could not accurately estimate S^{CD}_2. However, we have seen that in the case of DMPC and DPPC that this chain segment is very close to being parallel to the bilayer surface and, consequently, does not contribute very much to the chain length as estimated along the bilayer normal. At high temperatures and for all systems, the chain length does not seem to change much with a further increase in temperature. The difference in chain length among all systems at relative or absolute temperatures is of the order of 1.5 Å, i.e., is much less than the 2.5 Å expected to account for the 2-CH_2 unit difference between chains of DMPC and DPPC or DLPC. This clearly indicates the presence of *gauche* defects that shorten the projection of C-C bonds along the bilayer normal.

It is interesting here to compare results with DMPC with those obtained on the same system by Léonard (1993) using neutron diffraction on oriented samples. He found a hydrophobic thickness of 29.3 Å for DMPC at 50°C. The hydrophobic thickness is measured in this work from the ester region of one monolayer to the other ester region of the other monolayer. By taking twice the *sn*-2 $\langle L_{chain} \rangle$ value at 50°C, one finds 22 Å. Here one has to keep in mind that $\langle L_{chain} \rangle$ is a measure from C_1 to C_n. Unfortunately, the ester region is not well defined in the neutron Fourier profile because it accounts for O—C=O groups of both chains, which occupy different depths in the bilayer and, as a consequence, we cannot state clearly whether both methods lead to identical information. In addition, it is not clear whether twice the *sn*-2 chain is a correct estimate of the bilayer thickness. Work is currently being carried out with DMPC labeled in the *sn*-1 chain to compare both chain length and neutron data.

Validity of the model

In the following section, we will first discuss the accuracy and the method to calculate C-C bond order parameters. As shown in Theory, S^{CC} are calculated from experimental S^{CD}, (7), (8), and (9) and, thus, depend on the accuracy and on the assignment of C-D bond order parameters. However, the way to calculate S^{CC} begins with the end of the chain (9), i.e., in a region of the order profile where C-D order parameters are assigned and measured accurately, in the case of perdeuterated systems. The model seems to be robust enough because very similar profiles are equally obtained from perdeuterated systems (where positions in the *plateau* are not resolved) or from selectively labeled lipids. Therefore, there is no need for a very good resolution in the plateau region but, instead, a rather good one for positions near the chain end. This is exactly the situation encountered in perdeuterated systems, readily available. We are currently carrying out experiments with chains ^{13}C-labeled on two consecutive positions to compare the C-C bond order parameter as directly measured from ^{13}C-NMR with that obtained from ^2H-NMR with the model described herein.

Our method can be compared with that proposed by Schindler and Seelig (1975), where the average length of a chain composed of n segments is defined as

$$\langle L \rangle = 1.25 \left[n/2 - \sum_{i=1}^{n} S_i^{CD} \right].$$

In their model, derived from Marcelja's (Marcelja, 1974a, b), the calculation had been made for the *sn*-1 chain, and the contribution of the intermolecular motions was considered to be negligible. However, one may still try to compare the two methods by taking for instance DPPC data, at 41°C, from Table 3, summing the S_k^{CD} or S_k^{CC} from $k = 3$ to n (the inequivalence of deuterons at C_2 of the *sn*-2 chain is not accounted for in their model) and consider that $S_{mol} = 1$. One finds a value of 12.7 Å according to the Schindler and Seelig model and 13.4 Å according to ours. It must be mentioned that when using their model we considered that the total length of the C_{15}-C_{16} bond in the all-*trans* state is 2.2 Å (Schindler and Seelig, 1975). It is clear that the difference between both methods arises from the additional conformation (C in Fig. 2) that we include in our model to describe C-C ordering (13) and that is necessary to describe fully the isomerizations at a given carbon position. It is also interesting to test the validity of a more crude approach (Ipsen et al., 1990), where the chain length is defined as $\langle L \rangle = L_0 (1/2 + \langle S^{CD} \rangle)$. L_0 is taken as the chain length in the fully extended state, and $\langle S^{CD} \rangle$ is the average chain order parameter estimated from the first moment calculation (Davis, 1979) of a spectrum arising from a chain-perdeuterated sample. Measuring the first moment for [*sn*-2 $^2H_{31}$]DPPC at 41°C (Dufourc et al., 1986), one finds $M_1 = 57.6\ 10^3$ rad/s, which leads to $\langle S^{CD} \rangle = M_1 = 0.190$ and $\langle L \rangle = 13.6$ Å for the DPPC *sn*-2 chain length ($L_0 = 19.7$ Å). This must be compared with $\langle L_{chain} \rangle = 13.8$ Å we found by calculating the length from C_1 to C_n, using our model. In conclusion, the three approaches are very close, but ours leads to the largest value for $\langle L_{chain} \rangle$. We still have to postpone the final discussion about which of the models gives the closest length estimate, in comparison with neutron data, to our results on [*sn*-1-$^2H_{27}$]DMPC. We believe that our approach, which takes into account all conformers, the molecular order parameter contribution, and the inequivalence of order parameters when it occurs, is the more accurate.

CONCLUSION

C-C bond order parameters calculated from the S^{CD} order profile allow a quantitative description of the local ordering properties of the bilayer core. The studies described herein provide physical parameters such as the probabilities of segment conformations, the average acyl chain length, and the amount of *gauche* defects per chain. Our model is applicable to experiments carried out on perdeuterated systems, which are much easier to synthesize than several selectively chain labeled lipids. One of the strengths of our approach is that it can account for nonequivalent C-D bonds as for position C_2 of the *sn*-2 chain. The resulting S_2^{CC} brings information concerning the conformation of the beginning of the chain, and revealed that the *sn*-2 C_1-C_2 bond in DMPC and DPPC is not immobilized and has a tendency to occupy a position parallel to the bilayer surface. Acyl chain length measurements with our model revealed differences with previously published data. Calculations from first moments are in better agreement with our results than those directly made from the order profiles. This suggests that if one is not able to de-Pake spectra and use our approach, calculation from M_1 gives a good chain length estimate. However, the latter approach is restricted to very high quality powder spectra. Comparison of results with and without the contribution of S_{mol} does not reveal great differences in the acyl chain length calculation, when the molecular disordering is not important (e.g., near

T_C or in the presence of cholesterol). The very good agreement between our results on probabilities for each segment conformations and number of *gauche* defects per chain with those obtained from infrared experiments strongly supports our approach.

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APPENDIX

Expanding the Wigner matrix elements $D(\Omega)$ in terms of the reduced Wigner matrix elements $d(\theta)$ and exponential factors, (6) becomes:

$$S^{CC}_{k+1} = S_{mol}\{2\langle d_{02}(\theta_{isom})d_{20}(\theta_k)\cos(2\psi_{isom})\rangle + 2\langle d_{01}(\theta_{isom})d_{10}(\theta_k)\cos(\psi_{isom})\rangle + \langle d_{00}(\theta_{isom})d_{00}(\theta_k)\rangle\}. \quad (A1)$$

In the same way, one may express (5) as:

$$S^{CD}_k = S_{mol}\{2\langle d_{02}(\theta_{isom})d_{20}(\theta_k)\cos(2\psi_{isom} \pm 240°)\rangle + 2\langle d_{01}(\theta_{isom})d_{10}(\theta_k)\cos(\psi_{isom} \pm 120°)\rangle + \langle d_{00}(\theta_{isom})d_{00}(\theta_k)\rangle\}. \quad (A2)$$

Expanding the cosines leads to:

$$S^{CD}_k = S_{mol}\{-A_2 - A_1 + A_0 \pm \sqrt{3}\,B_2 \mp \sqrt{3}\,B_1\}, \quad (A3)$$

where

$$A_0 = \langle d_{00}(\theta_{isom})d_{00}(\theta_k)\rangle$$

$$A_1 = \langle d_{01}(\theta_{isom})d_{10}(\theta_k)\cos(\psi_{isom})\rangle$$

$$A_2 = \langle d_{02}(\theta_{isom})d_{20}(\theta_k)\cos(2\psi_{isom})\rangle$$

$$B_1 = \langle d_{01}(\theta_{isom})d_{10}(\theta_k)\sin(\psi_{isom})\rangle$$

$$B_2 = \langle d_{02}(\theta_{isom})d_{20}(\theta_k)\sin(2\psi_{isom})\rangle$$

and where the $\pm$ and $\mp$ signs stand for the respective deuterons on the same CD_2 unit. In cases where these two deuterons (R and S) are not equivalent, their respective S^{CD} order parameter can be written as:

$$S^{CD}_k(R) = S_{mol}\{-A_2 - A_1 + A_0 + \sqrt{3}\,B_2 - \sqrt{3}\,B_1\}$$
$$S^{CD}_k(S) = S_{mol}\{-A_2 - A_1 + A_0 - \sqrt{3}\,B_2 + \sqrt{3}\,B_1\}. \quad (A4)$$

Summation and use of (A1) leads to:

$$S^{CD}_k(S) + S^{CD}_k(R) = 3A_0S_{mol} - S^{CC}_{k+1}. \quad (A5)$$

Because the angle between a C-H bond and a C-C bond in a CH_2 group belonging to a hydrocarbon chain is $\theta_{isom} = 109.5°$ (Allinger et al., 1989), $d_{00}(\theta_{isom}) = -1/3$, which leads, with the help of (6), to:

$$S^{CD}_k(S) + S^{CD}_k(R) = -(S^{CC}_k + S^{CC}_{k+1}). \quad (A6)$$

It is trivial to show that when both deuterons are equivalent, $S^{CD}_k(S) = S^{CD}_k(R) = S^{CD}_k$, (A6) transforms to:

$$2S^{CD}_k = -(S^{CC}_k + S^{CC}_{k+1}). \quad (A7)$$

REFERENCES

Allinger, N. L., Y. H. Yuh, and J.-H. Lii. 1989. Molecular mechanics. The MM3 force field for hydrocarbons. *J. Am. Chem. Soc.* 111: 8551–8566.

Auguste, F., P. Barois, L. Fredon, B. Clin, E. J. Dufourc, and A. M. Bellocq. 1994. Flexibility of molecular films as determined by deuterium solid state NMR. *J. Physique II France.* 4:2197–2214.

Bloom, M., J. H. Davis, and A. L. MacKay. 1981. Direct determination of the oriented sample NMR spectrum from the powder spectrum for systems with local axial symmetry. *Chem. Phys. Lett.* 80:198–202.

Burnett, L. J., and B. H. Müller. 1971. Deuteron quadrupolar coupling constants in three solid deuterated paraffin hydrocarbons: C_2D_6, C_4D_{10}, C_6D_{14}. *J. Chem. Phys.* 55:5829–5831.

Casal, H. L., and R. N. McElhaney. 1990. Quantitative determination of hydrocarbon chain conformational order in bilayers of saturated phosphatidylcholines of various chain lengths by Fourier transform infrared spectroscopy. *Biochemistry.* 29:5423–5427.

Davis, J. H. 1979. Deuterium magnetic resonance study of the gel and liquid crystalline phases of dipalmitoylphosphatidylcholine. *Biophys. J.* 27: 339–358.

Davis, J. H. 1983. The description of membrane lipid conformation, order and dynamics by ^{2}H-NMR. *Biochim. Biophys. Acta.* 737:117–171.

Davis, J. H., K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs. 1976. Quadrupolar echo deuteron magnetic resonance spectroscopy in ordered hydrocarbon chains. *Chem. Phys. Lett.* 42:390–394.

Derbyshire, W., W. Gorwin, and T. Warner. 1969. A deuteron magnetic resonance study of a single crystal of deuterated malonic acid. *Mol. Physiol.* 17:401*a*. (Abstr.)

Dufourc, E. J., C. Mayer, J. Stohrer, G. Althoff, and G. Kothe. 1992. Dynamics of phosphate head groups in biomembranes. A comprehensive analysis using phosphorus-31 nuclear magnetic resonance lineshape and relaxation time measurements. *Biophys. J.* 61:42–57.

Dufourc, E. J., E. J. Parish, S. Chitrakorn, and I. C. P. Smith. 1984. Structural and dynamical details of cholesterol-lipid interactions as revealed by ^{2}H-NMR. *Biochemistry.* 23:6062–6071.

Dufourc, E. J., I. C. P. Smith, and J. Dufourcq. 1986. Molecular details of melittin-induced lysis of phospholipid membranes as revealed by deuterium and phosphorus NMR. *Biochemistry.* 25:6448–6455.

Dufourc, E. J., I. C. P. Smith, and H. C. Jarrell. 1983. A ^{2}H-NMR analysis of dihydrosterculoyl-containing lipids in model membranes: structural effects of a cyclopropane ring. *Chem. Phys. Lipids.* 33:153–177.

Dufourc, E. J., I. C. P. Smith, and H. C. Jarrell. 1984. Amphotericin and model membranes: the effect of Amphotericin B on cholesterol-containing systems as viewed by ^{2}H-NMR. *Biochim. Biophys. Acta.* 776: 317–329.

Engel, A. K., and D. Cowburn. 1981. The origin of multiple quadrupole couplings in the deuterium NMR spectra of the 2 chain of 1,2 dipalmitoyl-*sn*-glycero-3-phosphorylcholine. *FEBS Lett.* 126:169–171.

Flory, P. J. 1969. Statistical Mechanics of Chain Molecules. Interscience, New York.

Hauser, H., I. Pascher, R. H. Pearson, and S. Sundell. 1981. Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biochim. Biophys. Acta.* 650:21–51.

Ipsen, J. H., O. G. Mouritsen, and M. Bloom. 1990. Relationships between lipid membrane area, hydrophobic thickness and acyl chain orientational order. (The effects of cholesterol.) *Biophys. J.* 52:405–412.

Léonard, A. 1993. Influence de stéroides sur les propriétes dynamiques et structurales de membranes biologiques modèles. Une étude par RMN des solides et diffraction des neutrons. Ph.D. thesis. University of Bordeaux, France.

Léonard, A., and E. J. Dufourc. 1991. Interactions of cholesterol with the membrane lipid matrix. A solid state NMR approach. *Biochimie.* 73: 1295–1302.

Marcelja, S. 1974a. Chain ordering in liquid crystals. II. Structure of bilayer membranes. *Biochim. Biophys. Acta.* 367:165–176.

Marcelja, S. 1974b. Chain ordering in liquid crystals. I. Even-odd effect. *J. Chem. Phys.* 60:3599–3604.

Mayer, C., G. Gröbner, K. Müller, K. Weisz, and G. Kothe. 1990. Orientation-dependent deuteron spin-lattice relaxation times in bilayer membranes: characterization of the overall lipid motion. *Chem. Phys. Lett.* 165:155–161.

Mayer, C., K. Müller, K. Weisz, and G. Kothe. 1988. Deuteron NMR relaxation studies of phospholipid membranes. *Liquid Cryst.* 3:797–806.

Mehring, M. 1976. High-Resolution NMR Spectroscopy in Solids. Springer, Berlin.

Meier, P., E. Ohmes, and G. Kothe. 1986. Multipulse dynamic nuclear magnetic resonance of phospholipid membranes. *J. Chem. Phys.* 85:3598–3614.

Mendelsohn, R., M. A. Davies, H. F. Chuster, Z. Xu, and R. Bittman. 1991. CD_2 Rocking modes as quantitative Infrared probes of one-, two- and three-bond conformational disorder in dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylcholine/cholesterol mixtures. *Biochemistry.* 30:8558–8563.

Oldfield, E., R. Gilmore, M. Glaser, H. S. Gutowsky, J. C. Hshung, S. Y. Kang, T. E. King, M. Meadows, and D. Rice. 1978a. Deuterium nuclear magnetic resonance investigation of the effects of proteins and polypeptides on hydrocarbon chain order in model membrane systems. *Proc. Natl. Acad. Sci. USA.* 75:4657–4660.

Oldfield, E., M. Meadows, D. Rice, and R. Jacobs. 1978b. Spectroscopic studies of specifically deuterium labeled membrane systems. Nuclear magnetic resonance investigations of the effects of cholesterol in model membrane systems. *Biochemistry.* 17:2727–2740.

Paddy, M. R., F. W. Dahlquist, J. H. Davis, and M. Bloom. 1981. Dynamical and temperature dependent effects of lipid-protein interactions. Application of deuterium nuclear magnetic resonance and electron paramagnetic resonance spectroscopy to the same reconstitutions of cytochrome c oxidase. *Biochemistry.* 20:3152–3162.

Perly, B., E. J. Dufourc, and H. C. Jarrell. 1984. Facile and high yielding syntheses of phosphatidylcholines and phosphatidylethanolamines containing ^{2}H-labelled acyl chains. *J. Labelled Compd. Radiopharm.* 21:1–13.

Petersen, N. O., and S. Chan. 1977. More on the motional state of lipid bilayer membranes: interpretation of order parameters obtained from nuclear magnetic resonance experiments. *Biochemistry.* 16: 2657–2667.

Schindler, H., and J. Seelig. 1975. Deuterium order parameters in relation to thermodynamic properties of a phospholipid bilayer. A statistical mechanical interpretation. *Biochemistry.* 14:2283–2287.

Seelig, J. 1977. Deuterium magnetic resonance: theory and application to lipid membranes. *Q. Rev. Biophys.* 10:353–418.

Seelig, A., and J. Seelig. 1974. The dynamic structure of fatty acyl chains in a phosphatidylcholine bilayer measured by deuterium magnetic resonance. *Biochemistry.* 13:4839–4845.

Seelig, J., and W. Niederberger. 1974. Deuterium-labeled lipids as structural probes in liquid crystalline bilayers. A deuterium magnetic resonance study. *J. Am. Chem. Soc.* 96:2069–2072.

Senak, L., D. Moore, and R. Mendelsohn. 1992. CH_2 wagging progressions as IR probes of slightly disordered phospholipid acyl chain states. *J. Chem. Phys.* 96:2749–2754.

Spiess, H. W. 1978. Rotation of Molecules and Nuclear Spin Relaxation. Springer, Berlin.

Sternin, E., M. Bloom, and A. L. MacKay. 1983. De-Pakeing of NMR spectra. *J. Magn. Reson.* 55:274–282.

Stöhrer, J., G. Gröbner, D. Reimer, K. Weisz, C. Mayer, and G. Kothe. 1991. Collective lipid motions in bilayer membranes studied by transverse deuteron spin relaxation. *J. Chem. Phys.* 95:672–678.

Weisz, K., G. Gröbner, C. Mayer, J. Stohrer, and G. Kothe. 1992. Deuteron nuclear magnetic resonance study of the dynamic organization of phospholipid/cholesterol bilayer membranes: molecular properties and viscoelastic behavior. *Biochemistry.* 31:1100–1112.